Article Type: Invited Expert Review

Received Mar. 27, 2018 Accepted Apr. 16, 2018

Edited by: Alice Y. Cheung, University of Massachusetts, USA

Running title: Plant GPI-anchored proteins

**Title: Plant glycosylphosphatidylinositol (GPI) anchored proteins at the plasma membrane-cell wall
nexus**

Trevor H. Yeats^{1,2}, Antony Bacic^{3,4} and Kim L. Johnson^{3,4*}

1. School of Integrated Plant Sciences, Section of Plant Biology, Cornell University, Ithaca, NY 14853, USA

2. Robert W. Holley Center for Agriculture and Health, USDA-ARS, Cornell University, Ithaca, NY 14853, USA

3. Australian Research Council Centre of Excellence in Plant Cell Walls, School of BioSciences, University of Melbourne, Parkville, Victoria 3010, Australia

4. La Trobe Institute for Agriculture & Food, Department of Animal, Plant and Soil Sciences, La Trobe University, Bundoora, Victoria 3086, Australia

***Correspondence:** Kim L. Johnson (k.johnson@latrobe.edu.au)

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jipb.12659

This article is protected by copyright. All rights reserved

Abstract

Approximately 1% of plant proteins are predicted to be post-translationally modified with a glycosylphosphatidylinositol (GPI) anchor that tethers the polypeptide to the outer leaflet of the plasma membrane. While the synthesis and structure of GPI anchors is largely conserved across eukaryotes, the repertoire of functional domains present in the GPI-anchored proteome has diverged substantially. In plants, this includes a large fraction of the GPI-anchored proteome being further modified with plant-specific arabinogalactan (AG) *O*-glycans. The importance of the GPI-anchored proteome to plant development is underscored by the fact that GPI biosynthetic null mutants exhibit embryo lethality. Mutations in genes encoding specific GPI-anchored proteins (GAPs) further supports their contribution to diverse biological processes occurring at the interface of the plasma membrane and cell wall, including signaling, cell wall metabolism, cell wall polymer cross-linking, and plasmodesmatal transport. Here, we review the literature concerning plant GPI-anchored proteins in the context of their potential to act as molecular hubs that mediate interactions between the plasma membrane and the cell wall and their potential to transduce the signal into the protoplast and thereby activate signal transduction pathways.

Abbreviations: GPI-anchored proteins (GAPs), post-translational modifications (PTMs), phospholipase C/D (PI-PLC/D), arabinogalactan-protein (AGP), plasma membrane (PM), Golgi Apparatus (GA), endoplasmic reticulum (ER), receptor-like kinases (RLKs)

INTRODUCTION

Proteins associated with the plasma membrane are important structural components that also perform a myriad of essential functions in eukaryotes such as facilitating signaling, transport, and metabolism at the cell surface. Whilst many proteins are anchored and/or embedded within the plasma membrane (PM) by domains consisting of hydrophobic amino acid sequences, posttranslational attachment to lipids can also anchor proteins to membranes. On the intracellular face of the plasma membrane, myristoylation, prenylation and S-acylation are classes of post-translational modifications (PTMs) that can confer such associations (Hemsley 2015). On the other hand, the attachment of a GPI anchor during secretion of a protein targets the protein to the outer surface of the plasma membrane. The significance of the GPI anchor as a mode of surface display is thought to stem from two key aspects of its structure. First, a highly saturated lipid moiety can direct proteins to liquid-ordered microdomains, which act as foci for PM functions such as signal transduction. Secondly, cleavage of the GPI anchor can modulate protein function by allowing GPI-anchored proteins (GAPs) to diffuse into the extracellular

milieu and therefore participate in additional functions, such as cross-linking of wall polysaccharides/(glyco)proteins.

GAPs were first discovered based on their susceptibility to bacterial phosphatidylinositol-specific phospholipase C (PI-PLC) treatment. Treatment of mammalian cells with PI-PLC resulted in solubilization of specific isoforms of alkaline phosphatase, 5' nucleotidase, and acetylcholinesterase (Low 1989). Concurrently, GAPs were found in *Saccharomyces cerevisiae* (Conzelmann et al. 1988) and the parasitic protozoans *Trypanosoma brucei* and *Leishmania spp.* (Bordier et al. 1986). Several years later, the occurrence of GAPs in plants was demonstrated by similar means in a range of species (Morita et al. 1996; Kunze et al. 1997; Takos et al. 1997; Youl et al. 1998). With the availability of full genome sequences, computational prediction methods, and mass spectrometry-based proteomics in the first decade of the 21st century, the frequency and ubiquity of the GPI anchor became evident: approximately 1% of protein coding genes in eukaryotic genomes are likely to encode GAPs.

Dozens of studies on the structure of specific GPI anchors from diverse eukaryotic lineages has revealed a conserved glycan core with heterogeneity in the lipids and the substitutions (glycosyl and non-glycosyl) on the glycan core structure. The structure of the GPI anchor consists of a core glycan of three α -linked mannose (Man) residues and glucosamine (GlcN) linked to the C-terminal end of the polypeptide by phosphoethanolamine (**Figure 1A**). The lipid moiety is invariably attached to the glycan by *myo*-inositol, although the lipid itself can be either a glycerol- or sphingo-lipid. Elaborations of the core GPI structure can include either short mono-/oligo-saccharides or phosphoethanolamine residues attached to several positions on the core glycan (**Figure 1A**). Finally, palmitate attached to the C2 position of *myo*-inositol occurs in some mature GAPs. As discussed below, all GPI anchors transiently contain this additional lipid anchor, but it is usually removed during processing and secretion.

Despite the diversity of structures that have been observed in other kingdoms, to date, the sequence of only a single plant GPI anchor has been resolved. The arabinogalactan-protein (AGP) PcAGP1, isolated from *Pyrus communis* (pear) cell suspension culture, was shown to possess a GPI anchor with relatively simple structure devoid of phosphoethanolamine sidechains (Oxley and Bacic 1999). The only glycan modification to the conserved core was a β -linked galactose (Gal) sidechain on O4 of Man 1, which was detected with partial occupancy. The lipid moiety of PcAGP1 consists of a ceramide, as has been detected in several fungal GPI anchors (**Figure 1B**). A ceramide was also detected as the lipid component of the GPI anchor of an AGP isolated from *Rosa sp.* cell suspension culture (Svetek et al. 1999). There are no subsequent studies of GPI anchor sequences on any plant (glyco) protein.

Over the last two decades, most of the pathway of GPI anchor biosynthesis has been elucidated, primarily using cultured mammalian cells, protozoan parasites, and *S. cerevisiae* as models. In sum, the GPI biosynthetic pathway is largely conserved in these well-studied systems: Initially, the anchor is assembled on phosphatidylinositol in the ER and transferred *en bloc* to the processed C terminus of a protein (**Figure 2**). Subsequent remodeling of the anchor, particularly with regard to its lipid moiety, occurs during secretion. Approximately 30 genes have been identified to be involved in particular steps of GPI anchor assembly and their remodeling, and in most cases, homology between yeast and mammalian genes is evident. A survey of the *Arabidopsis* genome indicates most of these genes have single-copy homologs in plants (Luschnig and Seifert 2011), and mutation of five of these genes has experimentally supported their functional roles in GPI biosynthesis (**Figure 2**) (Lalanne et al. 2004; Gillmor et al. 2005; Dai et al. 2014; Bundy et al. 2016). Thus, the synthesis and core structure of GPI anchors appears to be conserved in plant and non-plant eukaryotes. This is not surprising as the evolution of GPI anchors likely predates the origin of eukaryotes and GAPs have been detected in several archaea species, and archaeal genome sequences also indicate a largely conserved biosynthetic pathway (Eichler and Adams 2005). What is still lacking in plants is complete characterization of the biosynthetic pathway to determine if all yeast and human orthologues are functional and if function is conserved. Structural determination of more plant GPI anchors is sorely needed to determine if variations in structure exist.

DELIVERY OF GAPs TO THE CELL SURFACE – ASSOCIATION WITH LIPID MICRODOMAINS

After GPI anchor attachment by a GPI transamidase to proteins synthesized in the ER lumen (Ellis et al. 2010; Kinoshita 2014) the secretion and further modification (glycosylation) of GAPs begins. GAPs are secreted through the ER and GA to the cell surface and there is evidence to suggest that this is selective rather than ‘bulk-flow’ (Muniz and Riezman 2000; Bonnon et al. 2010; Zurzolo and Simons 2016). Studies in plants, animals and fungi show GAPs associate with membrane domains rich in sphingolipids and sterols, termed lipid rafts or membrane microdomains. Depending on the organism, these start forming in the ER or Golgi apparatus (GA) and facilitate early sorting of GAPs as well as other membrane-associated proteins (Pielsticker et al. 2005; Bonnon et al. 2010). Remodeling of the GPI anchor after transfer onto the protein has been shown to be important in yeast and mammalian cells for association with lipids and sorting of GPI-anchored proteins (Fujita and Kinoshita 2012). In yeast, remodeling of the GPI-lipid occurs entirely in the ER and usually results in replacement with a very long highly saturated fatty acid such as ceramide (Muniz and Zurzolo 2014). In mammalian cells, remodeling

of the GPI-lipid begins in the ER with GPI-inositol deacylation. Unlike in yeast, subsequent fatty acid remodeling occurs at the GA (Muniz and Zurzolo 2014; Kinoshita and Fujita 2016). Transport to the GA involves p24 proteins, regulators of protein trafficking along the secretory pathway that associate with coat protein complex I (COPI) and COPII vesicles. In yeast, p24 proteins have been proposed to selectively export GAPs in vesicles distinct from other secretory proteins (Muniz and Riezman 2000; Castillon et al. 2011). In mammalian cells, p23 and p24 proteins, along with association with lipids, was found to concentrate GAPs at ER exit sites for packaging into distinct COPII vesicles and efficient ER-GA transport (Fujita et al. 2011). In *Arabidopsis* p24 proteins traffic between ER and GA and associate with ARF1 and coatamer (Montesinos et al. 2012; Montesinos et al. 2014), suggesting they also have roles in secretion. It is yet to be shown if p24 proteins are also involved in selected secretion of GAPs in plants.

Further sorting of GAPs in the GA is associated with differences in lipid composition of the cisternae. Sorting of GAPs in the GA has been characterized in polarized mammalian epithelial cells where they associate with sphingolipids and sterol rich microdomains that form apical sorting platforms (Lisanti et al. 1988). In the trans-golgi network (TGN), GAPs are segregated into distinct vesicles for delivery to the apical surface (reviewed in (Zurzolo and Simons 2016). Lipid asymmetry in the plasma membrane (PM) which is rich in sphingolipids in the outer leaflet and unsaturated phospholipids in the cytosolic leaflet directs apical or basolateral targeting. Association with lipids may not be the only mechanism for apical sorting in epithelial cells. Oligomerization of GAPs is also proposed to assist segregation from other proteins in the GA, enhance lipid ordering and membrane curvature to promote vesicle budding (Zurzolo and Simons 2016). The GAPs themselves could therefore also act as sorting signals for secretory and endocytic cargo at the TGN (Paladino et al. 2006). In animals, Golgi complexes are commonly located in the perinuclear region and are generally stationary, whereas in yeast, multiple Golgi compartments are found in either ordered stacks or as separated cisternae. Plant GAs are distinct again, being dispersed throughout the cytoplasm, mobile and consisting of a series of cisternae that are polarized into *cis*- (closest to ER), *medial*- and *trans*-cisternae (closest to TGN) (van de Meene et al. 2017). Sequential glycosylation, both *N*- and *O*-linked, occurs in the GA. As many plant GAPs are proposed to carry one, or both, of these PTMs they are presumed to be transported through the GA, however if this is via lipid microdomains is unclear.

Trafficking of GAPs in plants has only been characterized for a few proteins. Fusion of a secreted GFP variant to a GPI-anchored pectin methylesterase inhibitor protein (PMEI1) in tobacco showed it trafficked via the ER and GA before reaching the PM and cell wall (De Caroli et al. 2011). Interestingly, secretion of PMEI1 was found to be independent of the conventional secretory pathway.

Disruption of the GPI anchor signal sequence or blocking GPI anchor synthesis resulted in accumulation of PME11 in the Golgi stacks. Based on this study and others in plants where disruption of GPI anchor synthesis or attachment results in mis-localization (Gillmor et al. 2005; Dai et al. 2014; Xue et al. 2017), the GPI anchor may act as a sorting signal during secretion, similar to yeast and animals. The TGN in plants is suggested to have multiple sub-domains and mediate a diverse array of secretion pathways (van de Meene et al. 2017). Further studies of the secretion of a diverse range of plant GAPs is essential to determine if secretion is based on lipid association and/or other components. For example, many GAPs in plants contain *N*- and/or *O*-linked glycans that could reinforce transport to the outer leaflet of the PM due to protein-carbohydrate and/or carbohydrate-carbohydrate interactions within microdomains. Glycosyltransferases (GTs) involved in *O*-glycosylation have also been found in exocyst-positive organelles (EXPOs) that are part of the unconventional protein secretion pathway (Poulsen et al. 2014; Poulsen et al. 2015). This has led to the experimentally unverified suggestion that GPI-AGPs could be secreted via the EXPO pathway to direct targeted or polarized secretion (van de Meene et al. 2017).

Although it is assumed lipid asymmetry of the PM bilayer also exists in plants, only a few studies have attempted to demonstrate this. A study in oats suggested that, similar to animals, phospholipids and a minor galactolipid component typically predominate on the cytosolic leaflets of the PM. The apoplastic leaflet is enriched in sphingolipids and sterols (Tjellstrom et al. 2010). In plants GlycosylInositol PhosphorylCeramides (GIPCs) are the major sphingolipids and are enriched in the apoplastic face of the PM (Markham et al. 2013; Cacas et al. 2016). Studies in tobacco suggest poly-glycosylated GIPCs promote the formation of microdomains (Cacas et al. 2016) however, it is currently unknown if GAPs are present in GIPC-enriched microdomains. Targeted delivery to the PM and segregation of membrane regions into microdomains is proposed to facilitate co-existence of signaling partners, adhesion, matrix remodeling/interaction with cell wall components and pathogen responses (Konrad and Ott 2015).

Membrane microdomains have a more liquid-ordered state from the surrounding membrane, have reduced mobility and are resistant to solubilization with non-ionic detergents. This latter feature has enabled their isolation in 'detergent-resistant membranes' (DRMs) formed due to the aggregation of the lipid structures. Due to this aggregation, DRMs do not perfectly represent microdomains *in vivo* and enrichment of two proteins in these fractions is not sufficient evidence of *bona fide* co-location in the cell. Additionally, lack of a protein in DRMs does not exclude its association with microdomains *in vivo* as these could be lost during DRM preparation. Despite these caveats, this method, combined with proteomic techniques, is most widely used to study membrane microdomains and the GAPs that have come to characterize them in animals, yeast and plants. In plants, DRM proteomes have been determined

for numerous plant species including *Arabidopsis*, tobacco and rice (reviewed in (Takahashi et al. 2013; Tapken and Murphy 2015)).

GAPs consistently enriched in DRM proteomes include the fasciclin-like arabinogalactan-proteins (FLAs), Glycosyl Hydrolases family 3 (GH3) and 17 (GH17), Plastocyanin-like domain-containing proteins, skewed 5 (SKU5) and SKU5-similar (SKS) proteins, lipid transfer proteins (LTPs) and glycerophosphodiesterase-like proteins (GPDL/SHAVEN3-like proteins [SVL]) (Borner et al. 2005; Morel et al. 2006; Kierszniowska et al. 2009; Takahashi et al. 2012). These proteins largely overlapped with other proteomics approaches that involved enriching GAPs in membrane fractions and releasing them with phospholipase C or D (Borner et al. 2003; Elortza et al. 2006).

Characterizing the spatial resolution and functional partners of GAPs remains challenging. Advanced live-cell imaging techniques, such as super-resolution microscopy, have started to address the relationship between DRMs and microdomains. For example, investigation of the location of remorins, that associate with DRMs and are present on the inner leaflet of the PM, show that distinct sets of microdomains can occur (Jarsch et al. 2014; Konrad and Ott 2015). A number of studies also support the co-existence of proteins with similar functions in distinct microdomains. For example, PIN-formed (PIN) and P-glycoprotein (ABCB/PGP) regulators of auxin transport are proposed to stably associate in discrete microdomains in the PM to ensure coordinated auxin efflux (Titapiwatanakun et al. 2009). Few examples of plant GPI-anchored protein partners and co-location exist. In contrast, interaction of GAPs with components of the cell wall are becoming increasingly apparent. An excellent example of GAPs regulating the cell wall composition/organization in a targeted manner is shown by their role in plasmodesmata (PD).

ASSOCIATION OF GAPs WITH PLASMODESMATA IN A LIPID-DEPENDENT MANNER

PD are membrane-rich channels that traverse the cell wall and connect neighbouring cells to enable symplastic transport of small soluble proteins, RNA and solutes. PD therefore contain a distinct membrane for targeting of specific proteins and to generate a curved membrane organization (Mongrand et al. 2010; Bayer et al. 2014). Microdomains are suggested to occur in PD to restrict lateral movement and segregate PD proteins from the rest of the PM (Raffaele et al. 2009; Simpson et al. 2009; Fernandez-Calvino et al. 2011). The lipid composition of PDs is enriched in sterols and sphingolipids with very long chain saturated fatty acids (Grison et al. 2015). This is reminiscent of DRMs and consistent with this finding, members of the Remorin family and GAPs that are enriched in DRMs locate to PD (Raffaele et al. 2009; Fernandez-Calvino et al. 2011; Iswanto and Kim 2017). The sterol composition of PD was found to

be important for localization of two GPI anchored PD proteins, Callose Binding 1 (PDCB1) and β -1,3-glucanase (PdBG2) (Grison et al. 2015). In addition, delivery of PDCBP1 and PdBGs to PD was dependent on a GPI anchor (Grison et al. 2015; Zavaliev et al. 2016). These studies suggest that the specific membrane composition of PD is required for targeted secretion of GAPs to these sites. Interestingly, fusion of only the GPI anchors from PDCBP1 and pdBG2, as well as the GPI anchors from two non-PD localized proteins, AGP4 and LTGP1 to reporter proteins resulted in their enrichment in PDs. In non-PD located GAPs, the other functional domains within the protein are proposed to override the PD targeting (Zavaliev et al. 2016). This is reminiscent of the apical/basolateral sorting in epithelial cells and supports the notion that GPI anchors act as a preliminary sorting signal during secretion and in the GA/TGN other features in the protein either reinforce or redirect microdomain location.

Not only is the membrane surrounding PD specialized, but so is the cell wall that largely lacks cellulose and is rich in pectins and callose (Knox and Benitez-Alfonso 2014). Delivery of PDCBPs and PdBGs is required for their roles regulating callose deposition and cell-to-cell communication. Callose deposited in the neck regions of PD is believed to structurally constrict the PD aperture and therefore inhibit molecular trafficking. Some PDCBP members are predicted to have β -1,3-glucanase activity in addition to a callose binding X8 domain, providing a link between the PD and cell wall, important not only for PD opening but also for restricting lateral diffusion within the PD-PM.

GAPs within PD are also increasingly being recognized as having a crucial role in defense responses against fungal pathogens. The Lysin motif domain-containing GPI-anchored protein 2 (LYM2) is a chitin receptor-like protein responsible for changes in molecular flux through PD upon chitin perception (Faulkner et al. 2013). LYM2 is able to bind chitin oligosaccharides and acts independently of the receptor kinase for chitin signaling, CERK1 (Shinya et al. 2012; Faulkner et al. 2013). Enriched at PD, LYM2 was identified as a novel and critical component of pathogen-triggered responses and highlights the key role of GAPs mediating cell-to-cell communication through PD during pathogen perception. These examples emphasize the role of GPI-anchored attachment of proteins in the PM as an important step to enable specialized interactions with cell wall polymers.

MAKING THE PM – CELL WALL CONNECTION

In yeast, cross-linking of glycoproteins to cell wall glucans via transglycosylation is mediated by GAPs and is essential for normal development. In *S. cerevisiae* the wall is organized into multiple layers with cell wall proteins located in the outer layer and polysaccharides, largely β -1,6-glucan, β -1,3-glucan and chitin on the inner layers (Kapteyn et al. 1999; Free 2013). Cell wall glycoproteins, which carry *N*-

and/or *O*-linked glycans are often GPI-anchored and are essential for normal yeast cell wall integrity and morphology (Bowman et al. 2006; Pittet and Conzelmann 2007). GPI-anchored cell wall proteins are released from the wall after digestion by β -1,3-glucanase and β -1,6-glucanase digestion suggesting they were covalently linked to cell wall polysaccharides (Fleet and Manners 1977). GPI-anchored cell wall proteins were further shown to be covalently linked to β -1,6-glucan through phosphoethanolamine and several mannose residues (Kapteyn et al. 1997; Kollar et al. 1997; Fujii et al. 1999). Two GPI-anchored proteins with predicted GT activity, Defective Cell Wall 1 (DCW1) and Defective for Filamentous Growth 5 (DFG5) identified in *S. cerevisiae* were implicated in cleavage of the GPI anchor and transfer as mutants in these genes show weakened cell walls (Kitagaki et al. 2002). The enzymatic activity of DCW1 and DFG5 remains to be verified, however, their effects on cell wall integrity and importance for yeast viability is clear.

Transglycosylation of GAPs via their GPI anchors is not known to occur in plants, however, cross-linking of cell wall polysaccharides via transglycosylation is important for wall remodeling during growth (Frankova and Fry 2013). Interestingly, over 40% of the GAPs predicted by bioinformatics studies contain proteins with putative arabinogalactan (AG) or extensin-like glycosylation (Borner et al. 2003). These include classical AGPs, FLAs, plastocyanin-like AGPs (PAGs)/early nodulin-like and lipid transfer protein-like (LTPL) and receptor-like proteins involved in cell wall maintenance. These proteins are characterized by a domain rich in Proline (P) residues which can be hydroxylated to Hydroxyproline (Hyp, O) depending on the surrounding amino acids, and this acts as a signal for *O*-glycosylation (Kieliszewski and Shpak 2001; Zhao et al. 2002; Tan et al. 2003). In most GAPs, P residues occur in a non-contiguous manner, for example SPTP, such as occurs in AGPs, and this acts as a signal for glycosylation of large branched type II arabinogalactan (AG) polysaccharides (Tan et al. 2003). Covalent linking of AG to wall polysaccharides has been inferred in a number of plant species (Kjellbom et al. 1997) and has been confirmed for ARABINOXYLAN PECTIN ARABINOGLACTAN PROTEIN1 (APAP1) (Tan et al. 2013). APAP1 has been shown to be covalently cross-linked to both pectins and arabinoxylans. How universal AGP-wall polysaccharide interactions are remains unclear and is difficult to address due to the large heterogeneity of family members and the inability to identify them due to both glycosylation masking the protein backbones and the N-termini of many mature proteins being blocked by pyroglutamate (Johnson et al. 2017). AG glycans have also been suggested to act as ligands in signaling pathways due to the heterogeneity of their glycan structures. This intriguing association of GPI anchors with proteins proposed to contain AG glycans suggests plants have adapted unique mechanisms to make PM-cell wall connections. Further non-covalent interactions between GAPs and cell walls may be mediated by protein domains, for example, COBRA and COBRA-like GAPs have been shown to bind

cellulose via a carbohydrate-binding module (CBM)-like domain (see below; Li et al. 2013; Liu et al. 2013). Although it cannot be excluded that the GPI anchor covalently links to wall polysaccharides in plants, no clear DCW1 or DFG5 orthologues have been identified. It seems more likely therefore that GAPs associate with wall polysaccharides via protein/or *O*-glycan interactions and that release of the GPI from the PM occurs via another mechanism.

CELL WALL VS. PM LOCALIZATION

In animals, substrate-specific mechanisms have been proposed to release GAPs from the cell surface. Two classes of phosphatidylinositol-specific phospholipases (PI-PLs), PI-PLCs and PI-PLDs, are present in the ECM and are proposed to cleave the GPI anchor at different sites within the GPI moiety (Udenfriend and Kodukula 1995). For example, the GPI-specific PI-PLD in mammals is involved in cellular processes including adhesion, differentiation, proliferation, survival and oncogenesis (reviewed in Fujihara and Ikawa 2016). PI-PLCs are known to release GAPs in unicellular organisms. A study of *Paramecium* PI-PLCs show that in addition to a role in intercellular signaling pathways they can also be translocated to the cell surface to act on cell surface GAPs (Staudt et al. 2016). The role of PI-PLCs in multicellular organisms is less clear. A study of GAPs secreted by mammalian gastric tumor cells showed increased stability at the membrane in the presence of PI-PLC inhibitors (Song et al. 2006). This suggests that the function of PI-PLCs to release GAPs from the PM may have been conserved.

The mammalian protein GDE2 cleaves two different GAPs in order to regulate neurogenesis. GDE2 is anchored to the PM by 6 transmembrane helices with an extracellular catalytic domain. Although the protein was initially annotated based on sequence similarity to be a glycerophosphodiester phosphodiesterase, it was experimentally shown to catalyze the cleavage of the GPI-anchored protein RECK (reversion-inducing cysteine-rich protein with kazal motifs) by a PI-PLC-like mechanism (Park et al. 2013). Cleavage of RECK relieves its inhibition of ADAM (a disintegrin and metalloproteinase), a protease that releases peptide ligands that are perceived by the Notch receptor in a non-cell autonomous manner in order to promote neuronal differentiation. More recently, a second substrate of GDE2 was identified that is also involved in neurogenesis. The GPI anchor of heparan sulfate proteoglycan glypican-6 (GPC6) was also shown to be cleaved by GDE2. Although specific details of GPC6's role in neuronal differentiation are unclear, it is thought that this and other glypicans may play a role in this process by either recruiting and redistributing growth factors or acting as signaling ligands (Matas-Rico et al. 2016).

A second enzyme involved in substrate-specific GPI anchor cleavage by a different mechanism was recently identified in mammals. In contrast to GDE2, PGAP6 exhibits phospholipase A2 activity that

cleaves an acyl lipid group at the *sn*-2 position of glycerol, yielding a *lyso*-phosphatidylinositol-containing GPI anchor that is shed from the PM and further cleaved by extracellular phospholipase D activity (Lee et al. 2016a). PGAP6 was shown to exhibit substrate specificity for CRIPTO, a GPI-anchored co-receptor involved in embryo development, as the GPI anchor of its close homolog CRYPTIC was not processed (Lee et al. 2016a).

To date, no GPI-specific phospholipases have been characterized from plants although there are many candidates with sequence similarity to phospholipases and the requisite localization to the secretory system, PM, and/or extracellular space. The structural characterization of the GPI anchor present on the soluble isoform of *Pyrus communis* AGP1 indicated the absence of a terminal phosphate, suggesting that this GPI lipid was cleaved either by a phospholipase D or a phospholipase C with subsequent phosphatase action (Oxley and Bacic 1999). The identification of both specific and general GPI cleavage mechanisms in plants and their significance is an interesting area of future study.

A number of GAPs present in DRMs could be released by treatment with PI-PLC supporting the idea that their PM location could be regulated by the action of PI-PLs (Borner et al. 2003; Lalanne et al. 2004). Visualization of GAP release *in vivo* is needed to confirm such events yet very few examples exist. Using immuno-gold labelling of a COBRA-like protein in rice, BRITTLE CULM 1 (BC1), the authors showed BC1 located to the PM in cells with thin walls, and into the cell wall in cells with thickened, secondary walls (Liu et al. 2013). BC1 was also shown to be PI-PLD sensitive. Similarly, the GAP ZERZAUST (ZET) localized not to PM but to the cell wall (Vaddepalli et al. 2017). A number of studies have also shown that although removing the GPI-signal sequence disrupts trafficking to the surface, limited release of protein into the cell wall can still occur and, interestingly, recover protein function when used in complementation studies (Liu et al. 2016; Vaddepalli et al. 2017; Xue et al. 2017). The activity of PI-PLs is therefore likely to influence the release and function of GAPs. Further work is needed to understand the temporal and spatial dynamics of GAP release in response to PI-PL activity.

THE GPI-ANCHORED PROTEOME - PREDICTION OF GPI ANCHOR SIGNALS

The pro-sequence of GAPs typically contains two signal sequences at their N- and C- termini that are both cleaved in the mature GPI-anchored protein (Figure 1A). The N-terminal peptide is a typical secretory signal peptide that is recognized for SRP-dependent co-translational insertion of the polypeptide into the endoplasmic reticulum (ER). Eukaryotic secretory signal peptides are typically 15-30 amino acids in length, and while the amino acid sequence is not conserved, the general pattern of charged and hydrophobic amino acids in these peptides make their prediction by machine learning-based computer

programs such as SignalP straightforward (Petersen et al. 2011). While approximately 10% of the plant proteome contain such signal peptides, only 10% of these are potentially further modified by attachment of a GPI anchor as specified by their C-terminal signal peptides.

The C-terminal signal peptide of GAPs is recognized by the GPI-transamidase complex, which cleaves the peptide at a site designated as the ω site and attaches the GPI anchor to the protein via an amide linkage. Although the specific sequence around this site is not conserved, significant features have been characterized. First, the amino acid sequence from $\omega-11$ to $\omega-1$ is typically unstructured. The region surrounding the ω site, from $\omega-1$ to $\omega+2$ tends to be composed of small residues. This is followed by a spacer region ($\omega+3$ to $\omega+9$) of moderate polarity and finally a hydrophobic region that spans the remaining peptide sequence. Based on these features and training sets of *bona fide* GAPs, a number of computer programs have been developed for genome-wide prediction of GPI anchor C-terminal signal sequences. These include BIG-PI (Eisenhaber et al. 1999), PredGPI (Pierleoni et al. 2008), GPI-SOM (Fankhauser and Maser 2005), and FragAnchor (Poisson et al. 2007). However, at present, these methods are universally limited by false positive rates that are of the same magnitude as the genome-wide frequency of genes that encode GAPs. Thus, published genome-wide censuses of predicted GAPs, such as Borner et al. (2003), are likely incomplete in addition to containing a number of false positives. For example, Borner et al. (2003) list At4g28560 (also known as Rac-interactive binding motif-containing protein 7 [RIC7]) as a predicted GAP, although further characterization of this protein indicated dynamic relocalization between the nucleus and plasma membrane that is not characteristic of GAPs (Jeon et al. 2008). A further complication is suggested by the possibility of GAPs without signal peptides that are “double anchored” by internal transmembrane sequences. For example, the *Arabidopsis* gene *NON RACE-SPECIFIC DISEASE RESISTANCE 1* (*NDRI*) encodes a protein with two predicted transmembrane domains, although the mature protein’s C terminus is cleaved and contains inositol, a likely marker of GPI anchor attachment (Coppinger et al. 2004). However, as this protein is not released from membrane fractions by treatment with PI-PLC and it does not contain a signal peptide, it would not be detected by most experimental or computational approaches for identifying GAPs. Thus, while the majority of the GPI-anchored proteome can be predicted, some of the most intriguing cases may escape high throughput experimental or computational detection.

THE PLANT GPI-ANCHORED PROTEOME

Despite the conservation of GPI biosynthetic processes across eukaryotes, there are no obvious GPI-anchored protein homologs shared between the different kingdoms of eukaryotic life. However, within the green plant lineage, the repertoire of GAPs is generally well conserved, with many GPI-anchored protein-encoding genes forming gene families within each given species. While the majority of experimental evidence with regard to GPI-anchored protein function is from the *Arabidopsis* system, there are several examples of homologous genes having similar functions in other species. A study of the hydroxyproline-rich glycoproteins (HRGP) superfamily in the 1000 plant transcriptomics data (1KP) showed that GPI anchored AGPs (GPI-AGPs) can be identified early in land plant lineages, being present in Glaucophyta, Chromista and green algae (Johnson et al. 2017) and were found throughout land plant evolution in all 1KP groups. Evolutionary studies of chimeric AGPs, many of which are predicted to be GPI anchored, could also be identified in the genomes of 47 plant species (Ma et al. 2017). In addition, studies of GPI-LTPs, proposed to function in the synthesis and/or deposition of cutin and cuticular waxes, in the moss *Physcomitrella* suggest they share similar features and function to GPI-LTPs found in flowering plants (Edstam and Edqvist 2014; Edstam et al. 2014).

Phylogenetic studies suggest that individual GPI-AGP members can be traced throughout evolution suggesting conservation of function. For example, orthologues of the *Arabidopsis* pollen-specific *AGP6* and *AGP11* (Coimbra et al. 2009) could be robustly identified in samples that contained floral tissue in angiosperms (Johnson et al. 2017). Similarly, the GPI-anchored AG peptide, *AGP23* has been shown to be predominantly expressed in pollen in *Quercus suber* (cork oak), *Prunus persica* (peach) and *Populus trichocarpa* (Western balsam poplar) (Costa et al. 2015). These studies suggest that, at least in angiosperms, the function of GPI-AGPs and other GAPs may be conserved in reproductive development.

FUNCTION OF PLANT GAPs

Loss of GPI-anchoring in plants results in lethality, either gametophytic or embryonic. For example, mutations in the *Arabidopsis* homolog of mammalian PIG-M, an ER-localized mannosyltransferase required for synthesis of GPI anchors show defects in cell wall synthesis in the embryo and is lethal (Gillmor et al. 2005). Phenotypes associated with specific GAPs in plants are summarized in Table 1. These studies highlight the role of GAPs in plant development, and we provide specific examples of two key areas that are emerging, roles in signaling and cell wall maintenance.

CELL WALL INTEGRITY MAINTENANCE AND SIGNALING?

Although proposed for many years based on the signaling function of GAPs in other organisms, direct interaction of GAPs in plants with signaling proteins has only recently been experimentally shown. An elegant example is in the female gametophyte, where LORELEI (LRE), a GAP expressed exclusively in the ovule functions in female fertility (Liu et al. 2016). A related protein LRE-like GAP (LLG1) is more universally expressed and mutants show defects in seedling development. The similarity of *llg1* mutants phenotypes to that of mutants in FERONIA (FER), a receptor-like kinase implicated in cell wall integrity sensing, suggested they may act in the same pathway (Huck et al. 2003; Wolf and Hofte 2014; Li et al. 2015). Investigation of double mutants of *llg1* and *fer* confirmed a genetic interaction. Both LLG1 and LRE are essential for membrane localization of FER, with FER-GFP located to ER in *llg1* and *lre* mutants. LLG1 and LER physically interact with FER and are proposed to act as molecular chaperones to regulate trafficking and modulate its activity in multiple signaling pathways at the PM (Li et al. 2015). Further research investigating the specific interaction between LRE and FER in the filiform apparatus of synergid cells showed they jointly function in pollen tube reception and the GPI anchor of LRE was required for its localization in the filiform apparatus but not its function (Liu et al. 2016). Recently, an additional role of LRE-like proteins in defense signaling has been demonstrated. LLG1 forms complexes with FLAGELLIN SENSING 2 (FLS2), the elongation factor-Tu receptor (EFR), and BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) in order to regulate innate immunity in response to pathogen-associated molecular patterns (PAMPs; (Shen et al. 2017)).

Also shown to interact with FER is ENOD-like 14 (ENODL/EN14). Containing a plastocyanin-like domain, AG glycomotifs and GPI anchored, ENODLs 11-15 are predominantly expressed in ovules and accumulate at the filiform apparatus (Hou et al. 2016). Reduced levels of *enod11-15* through RNAi resulted in reduced pollen tube rupture and attraction of multiple pollen tubes. Potentially working in a larger FER complex, further elucidation of ENODLs, LRE and LLG1 in signaling pathways and the importance of GPI anchoring in this process is required.

The role of GAPs in pollen tube growth is also exemplified by disrupting SETH1 and SETH2, involved in the first step of GPI biosynthetic pathway. Loss of SETH1 and SETH2 severely affected pollen germination efficiency and pollen tube growth, likely associated with altered callose deposition (Lalanne et al. 2004). A combined transcriptomics and proteomics approach to identify putative pollen-specific GAPs that might be disrupted in *seth1* and *seth2* detected β -1,3-glucanases, phytoacyanins, FLAs

and LTPs, many of which have members implicated in regulating cell walls. Reminiscent of PD, a specialized wall and membrane occurs in pollen tubes for growth that encompasses significant elongation whilst maintaining mechanical strength (Hepler et al. 2013; Ischebeck 2016). GAPs appear to have evolved important roles in maintaining both cell wall integrity and signaling during pollen tube growth and reception.

The link with PD and floral development is further emphasized by investigation of ZERZAUST (ZET), a GAP with similarity to β -1,3-glucanases (Vaddepalli et al. 2017). ZET likely acts together or in parallel with the atypical receptor-like kinases (RLKs) STRUBBELIG and QUIRKY, both localized to PD, to regulate development of ovules, flowers, stems and root hairs. Although localized to the cell wall, ZET is shown not to act on callose but rather acts non cell-autonomously to influence cell wall composition. ZET is potentially a mobile extracellular signaling protein that has a regulatory role controlling cell wall biology and tissue morphology.

In the seed coat, another glycoprotein, FLA4/SOS5 is proposed to act in the same pathway as two leucine-rich repeat receptor like kinases, FEI1 and FEI2, through genetic interaction studies of the mutants (Basu et al. 2016; Showalter and Basu 2016). Basu et al., 2016 suggest they physically interact through the AG glycans on FLA4, however this has not been biochemically confirmed. FLA4 lacking *O*-glycan structures was still able to complement a *fla4* mutant and FLA4 could function as a soluble extracellular factor suggesting it may only transiently act as a ligand for RLKs (Xue et al. 2017). The association of proteins with AG glycans and GPI anchors in plants suggests that they may play multiple roles. The potential for AG glycans to cross-link to other cell wall polymers and influence the wall matrix supports this view (see earlier section). In addition, FLA11 and FLA12 in *Arabidopsis* and their orthologues in other species have been shown to influence the microfibril angle of cellulose in secondary walls (MacMillan et al. 2010; MacMillan et al. 2015). In *fla11 fla12* mutants, this is proposed to result in changes to stem biomechanical properties (MacMillan et al. 2010). Further work is needed to investigate GAPs with AG glycans and their proposed multifunctional roles in maintenance and perception of wall integrity.

GAPs AND CELL WALL BIOSYNTHESIS AND MAINTENANCE

GAPs that act to modify cell wall polymers are well characterised in fungi. For example, two putative GPI-anchored glycoside hydrolases, BGT-1 and BGT-2, in *Neurospora crassa* are proposed to be important for cell wall re-modelling at the hyphal dome and during conidiation to ensure controlled growth and cell wall assembly (Martinez-Nunez and Riquelme 2015). In plants, callose binding GPI-

anchored glucanases are involved in modifying the cell walls of PD (see above). In addition, GAPs that act on other wall polymers, such as xyloglucan and cellulose are also known to exist and can be involved in modulating both the early synthesis and later re-modelling of polysaccharides.

Cellulose is the main structural component of the plant cell wall, forming strong microfibrils (MFs) most likely composed of 18-24 β -(1,4)-glucan chains. Synthesized by cellulose synthases (CESAs) at the PM, a number of proteins that associate with the CESA complexes (CSCs) can alter cellulose biosynthesis, for example, by regulating the activity of CESAs or the crystallization of MFs. It is proposed that CSCs have a specialized membrane environment due to the high levels of S-acylation on CESAs and their hydrophobicity (Kumar et al. 2016). Similar to GPI-addition, S-acylation is known to facilitate microdomain formation and/or protein partitioning (Konrad and Ott 2015). A known CESA interacting protein, KORRIGAN, has also been shown to be S-acylated and it is possible that GAPs, such as COBRAs, partition into CSCs microdomains to form large regulatory complexes (Hemsley et al. 2013).

Members of the COBRA-like family of GAPs are known to regulate the deposition of cellulose into the wall (Li et al. 2013; Ben-Tov et al. 2015). A COBRA-like protein, BC1 has been shown to directly bind cellulose to affect MF crystallinity (Liu et al. 2013). A subset of COBRAs appear to be specialized for function in secondary cell walls, which typically consist of 40-50% cellulose and are thickened to provide rigidity (Zhong and Ye 2015). COBRAs are therefore implicated in regulating wood and fiber quality (Gritsch et al. 2015; Niu et al. 2015). Loss of COBRA-like protein activity has been shown to cause plant growth defects, such as dwarfing and reduced tiller number in the *brittle culm* mutants in rice (Dai et al. 2011; Liu et al. 2013), altered leaf architecture in tobacco (Gao et al. 2013), disrupted seed coat mucilage (Ben-Tov et al. 2015) and pollen tube guidance (Li et al. 2013) in *Arabidopsis*. In pollen tubes, COBRA-like 10 (COBL10) is localized at the most apical region, where newly synthesized cell wall material is deposited, and this localization is dependent on an intact GPI anchor. COBL10 is proposed to regulate organization of pectins in the wall in response to sensing female signals. Recently, two putative GPI-anchored aspartic proteases, A36 and A39, were shown to co-localize with COBL10 (Gao et al. 2017a; Gao et al. 2017b). In the apical cell walls of pollen tubes in double *a36 a39* mutants, increased levels of highly methyl esterified homogalacturonan pectins and xyloglucans were detected. GPI-anchored aspartic proteases in yeast have also been shown to function in cell wall integrity and/or remodeling (Krysan et al. 2005; Kaur et al. 2007).

GAPs may also be important for defense against fungal pathogens by modulating the cell wall and limiting penetration. For example, *PMR6* encodes a GPI-anchored pectate lyase protein involved in pectin degradation and potentially release of pectic oligosaccharides. Increased pectin content in epidermal cell walls of *pmr6* mutants has been shown to increase resistance against powdery mildew and

Colletotrichum higginsianum (Vogel et al. 2002; Engelsdorf et al. 2017). It is proposed that this may result in higher mechanical resistance during infection or alter signaling. Despite being only a minor component of the extracellular matrix, the conservation of roles for GAPs fine tuning the cell wall for growth, development, defense and signaling emphasizes their importance.

CONCLUSIONS AND FUTURE DIRECTIONS

Twenty years after their discovery in plants there are many remaining questions as to the function of GAPs. Recent work has assigned biological significance to dozens of these proteins by way of a range of mutant phenotypes. We have attempted to highlight the broad significance of various GAPs to the biology of plants. The long list of essentially ‘unknown proteins’ that constitute the plant GPI-anchored proteome as described by Borner and others in the early 2000’s now has mutant phenotypes associated with many of these genes. However, the biochemical function of most GAPs remains obscure but with advances in both cell/molecular techniques and modern instrumentation should now be tractable. Notably, many plant GAPs are spuriously annotated based on sequence similarity to biochemically characterized proteins, although closer examination often reveals a loss of conserved amino acids involved in ligand binding (Sedbrook et al. 2002) or catalysis (Hayashi et al. 2008; Yeats et al. 2016). As many plant GAPs are encoded by gene families that are taxonomically conserved, neo-functionalization likely occurred early in the evolutionary history of green plants. An intriguing area for future study is the biology of GAPs in early diverging lineages of green plants, such as the charophycean green algae (Domozych et al. 2016).

Another consequence of plant GAPs being encoded by gene families is that redundancy may obscure phenotypic effects of single gene mutations. For example, disruption of five genes by a combination of RNA interference and T-DNA insertion was required to demonstrate the role of EARLY NODULIN-like proteins in pollen tube reception (Hou et al. 2016). Multiplexed targeted mutagenesis by CRISPR is likely to facilitate the generation of such higher order mutants, both in *Arabidopsis* and in other plant species (Cermak et al. 2017).

While analysis of mutant phenotypes provides strong evidence for the significance of GAPs to specific biological processes, deeper mechanistic understanding will come from the identification of physical protein interactions. GAPs, like other secreted proteins, are recalcitrant to high-throughput methods such as yeast two hybrid screens. In several cases, co-immunoprecipitation has been used to demonstrate *in vivo* protein-protein interactions of specific GAPs (Li et al. 2015; Hou et al. 2016; Shen et al. 2017). As discussed earlier, intermolecular interactions involving GAPs are likely to be facilitated in

part by lipid interactions that involve the GPI anchor. New technologies for detergent-free solubilization of membrane-protein complexes in their native lipid environment may be a means of directly testing this and improving the specificity of co-immunoprecipitation experiments. For example, linear copolymers of styrene and maleic acid form amphiphilic loops that can solubilize two dimensional discs of membranes and associated proteins that are termed styrene maleic acid-lipid particles (SMALPs). SMALPs behave essentially as soluble protein-lipid complexes that are amenable to downstream biochemical purification and characterization (Lee et al. 2016b).

To date, the structure of the GPI anchor has only been resolved for a single, highly abundant plant GPI-anchored protein, PcAGP1 (Oxley and Bacic 1999). Results from other systems suggest that a diversity of GPI anchor structure should be expected (Tsai et al. 2012). The increased sensitivity and resolution of mass spectrometry are likely to facilitate further characterization of plant GPI anchor structure. It will be interesting to see whether the relatively simple structure of PcAGP1's anchor is typical, or if glycan and PEtN sidechains are frequent modifications of other GPI anchors in plants as they are in other systems. Notably, putative orthologs of genes encoding enzymes involved in the attachment of PEtN sidechains are present in plant genomes (Luschnig and Seifert 2011). Another point that can be addressed by further characterization of plant GPI structures is the nature of the lipid groups. In the GPI anchor of PcAGP1, the lipid group was shown to be a ceramide consisting primarily of phytosphingosine and tetracosanoic acid (Oxley and Bacic 1999). The predominant form of ceramide in GPI anchors in suspension cultured cells of rose (*Rosa* sp.) was composed of tetracosanoic acid and 4-hydroxysphinganine (Svetek et al. 1999) suggesting some variation exists. Whether sphingolipids like this are a universal feature of plant GPI anchors, or if other lipids might confer unique properties to the GPI anchor is an intriguing area for future investigation.

As the GPI-anchored proteomes of plants appear to be distinct from other eukaryotes, it will be interesting to see how plants have adopted this post translational modification to function in the many biological processes occurring at the interface of the plasma membrane and cell wall. Many aspects of growth, development, and signaling ultimately converge here, and genetic evidence indicates the essential contribution of GAPs to all of these processes. We suggest that research efforts should now focus on the definition of what aspects of GPI biology are distinctive or uniquely important to plants. Technical advances in microscopy, mass spectrometry, and genome editing now provide the means to address these questions experimentally. Understanding how GAPs regulate signaling and cell wall metabolism will enable us to better understand their roles in fine tuning plant growth and development.

ACKNOWLEDGEMENTS

AB and KLJ were supported by the ARC Centre of Excellence in Plant Cell Walls grant [CE1101007]. THY was supported by a postdoctoral fellowship from the Philomathia Foundation.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare

AUTHOR CONTRIBUTIONS

T.H.Y., A.B. and K.L.J. planned and wrote the article.

Table 1. Plant GPI-anchored proteins and respective mutant phenotypes

Name	Locus ID(s)	Gene family	Phenotype/notes	Reference
<i>Arabidopsis thaliana</i>				
ATBG_PPAP	AT5G42100	Beta-glucosidase	Control of symplastic connectivity	(Levy et al. 2007)
AGP4	AT5G10430	Classical AGP	Synergid degeneration	(Pereira et al. 2016)
AGP6/AGP11	AT5G14380/ AT3G01700	Classical AGPs	Pollen development	(Coimbra et al. 2009)
AGP17	AT2G23130	Classical AGP	Biotic responses	(Gaspar et al. 2004)
AGP18	AT4G37450	Classical AGP	Female gametogenesis and megaspore selection	(Acosta-Garcia and Vielle-Calzada 2004; Demesa-Arevalo and Vielle-Calzada 2013)
A36 and A39	AT5G36260 AT1G65240	Aspartic protease	Pollen and ovule development	(Gao et al. 2017a)
COBRA	AT5G60920	COBRA-like	Primary wall cellulose	(Schindelman et al. 2001)
COBRA-like 10	AT3G20580	COBRA-like	Pollen tube	(Li et al. 2013)
COBRA-like 2	AT3G29810	COBRA-like	Seed coat mucilage	(Ben-Tov et al. 2015)
COBRA-like 4/IRX6	AT5G15630	COBRA-like	Secondary wall cellulose	(Brown et al. 2005)
COBRA-like 9	AT5G49270	COBRA-like	Root hair development	(Jones et al. 2006)

ENODL11-15	AT2G23990 AT4G30590 AT5G25090 AT2G25060 AT4G31840	Early-nodulin like/plastocyanin	Pollen tube reception	(Hou et al. 2016)
FLA1	AT5G55730	Fasciclin-like AGP	Reduced shoot regeneration in tissue culture	(Johnson et al. 2011)
FLA11 and FLA12	AT5G03170 AT5G60490	Fasciclin-like AGP	Secondary cell wall mechanics and cellulose	(MacMillan et al. 2010)
FLA3	AT2G24450	Fasciclin-like AGP	Microspore development, cellulose deposition defect	(Li et al. 2010)
FLA4/SOS5	AT3G46550	Fasciclin-like AGP	Cell expansion; salt sensitivity	(Shi et al. 2003)
FLA9	AT1G03870	Fasciclin-like AGP	Seed abortion under drought stress	(Cagnola et al. 2018)
LORELEI	AT4G26466	Lorelei-like	Pollen tube reception	(Capron et al. 2008)
LLG1	AT5G56170	Lorelei-like	Rapid alkalization factor (RALF) perception	(Li et al. 2015)
LTPG	AT1G27950	Lipid-transfer protein	Cuticular wax export	(Debono et al. 2009)
LTPG2	AT3G43720	Lipid-transfer protein	Cuticular wax export	(Kim et al. 2012)
LYM1 and LYM3	AT1G21880 AT1G77630	Lysin-motif (LysM)-domain containing	Peptidoglycan sensing	(Willmann et al. 2011)
NDR1	AT3G20600	Late embryogenesis abundant (LEA) hydroxyproline- rich glycoprotein family	Mediates SAR, possibly double-anchored	(Coppinger et al. 2004)
PdBG1 and PdBG2	AT3G13560 AT2G01630	Beta-glucosidase	Control of symplastic connectivity, lateral root	(Benitez-Alfonso et al. 2013)

			formation	
PMR6	AT3G54920	Pectate-lyase-like	Powdery mildew resistance	(Vogel et al. 2002)
SHAVEN3	AT4G26690	SHAVEN3-like	Root hair defective	(Jones et al. 2006)
SHAVEN3-like 1	AT5G55480	SHAVEN3-like	Cellulose-deficient and sucrose-sensitive (in combination with SHV3)	(Hayashi et al. 2008; Yeats et al. 2016)
SKU5	AT4G12420	skewed 5-like/cupredoxin-like	Root skewing	(Sedbrook et al. 2002)
XYP1 and XYP2	AT5G64080 AT2G13820	Lipid-transfer protein (xylogen)	Promotion of xylem differentiation, vascular development defects in mutant	(Motose et al. 2004)
ZERZAUST	AT1G64760	Beta-1,3-glucanase	Aberrant cell morphogenesis	(Vaddepalli et al. 2017)
<i>Oryza sativa</i>				
SRL1/CLD1	Os07g01240	Unknown	Curled leaves, dwarf, reduced cellulose, enhanced water loss	(Xiang et al. 2012)
BRITTLE CULM 1	Os03g30250	COBRA-like	Secondary cell wall synthesis	(Li et al. 2003; Liu et al. 2013)
<i>Zea mays</i>				
BRITTLE STALK 2		COBRA-like	Secondary cell wall synthesis	(Ching et al. 2006; Sindhu et al. 2007)

Figure Legends

Figure 1. Structural features of GPI-anchored proteins

(A) The nascent polypeptide of proteins that will be GPI-anchored include a N-terminal secretory signal peptide (SP) and a C-terminal GPI-specifying hydrophobic signal sequence (SS) that are cleaved in the ER during maturation. Approximately 40% of plant GPI-anchored protein encoding genes also contain predicted arabinogalactan (AG) glycomodule domains, and mature GPI-anchored proteins may also contain N-linked glycans. The general structure of the GPI anchor with glycosyl linkages is shown. Variable structural features that have been observed in a range of eukaryotic species are indicated with parentheses. (B) The structure of *Pyrus communis* AGP1 GPI anchor as reported by Oxley and Bacic (1999). Aside from the core GPI glycan structure, only a β-1,4-linked Gal sidechain was observed with 0.54 occupancy, as indicated. The lipid was found to be a ceramide consisting primarily of phytosphingosine and tetracosanoic acid, with minor isoforms consisting of 4-hydroxy-8-sphingenine and/or docosanoic acid. Other abbreviations used are Man = mannose, PetN = phosphoethanolamine, Gal = galactose, GalNAc = N-acetyl galactosamine, GlcN = glucosamine, PI-PLC = phosphatidylinositol-specific phospholipase C, PI-PLD = phosphatidylinositol-specific phospholipase D, PLA2 = Phospholipase A2.

Figure 2. Biosynthesis of GPI-anchored proteins in plants

This scheme is based on the pathway as elucidated in mammalian, protozoan and yeast systems. GPI anchor biosynthesis is initiated by generation of *N*-glucosamine-phosphoinositide (steps 1-2) on the cytoplasmic surface before flipping by an unknown mechanism (proposed to be a “flippase”) to the ER lumen (step 3). Following acylation of inositol (step 4) and synthesis of the trimannosyl core (steps 5-7), elaboration of the GPI-anchor in plants is proposed to include galactosylation (step 8) and potentially addition of PEtN sidechains (not shown). A GPI transamidase complex transfers the GPI anchor to the protein (step 10) before export from the ER. Inositol deacylation likely occurs before plant GPI-anchored proteins reach the cell surface, but it is unknown if further fatty acid remodeling occurs as in yeast, protozoa and mammals. Most yeast and mammalian GPI biosynthetic genes have single copy orthologs in the *Arabidopsis thaliana* genome, and mutants deficient in five of these genes have been characterized (*seth1*, *seth2*, *pnt1*, *aptg1*, and *atgpi8*). The orthologous human (PIG) and yeast (Gpi) proteins are indicated. *Arabidopsis* genes putatively involved in GPI biosynthesis based on sequence homology are outlined in Luschnig and Seifert (2011) and Ellis et al. (2010).

REFERENCES

- Acosta-Garcia G, Vielle-Calzada JP (2004) A classical arabinogalactan protein is essential for the initiation of female gametogenesis in *Arabidopsis*. **Plant Cell** 16: 2614-2628
- Basu D, Tian L, Debrosse T, Poirier E, Emch K, Herock H, Travers A, Showalter AM (2016) Glycosylation of a Fasciclin-Like Arabinogalactan-Protein (SOS5) Mediates root growth and seed mucilage adherence via a cell wall receptor-like kinase (FEI1/FEI2) pathway in *Arabidopsis*. **PLoS ONE** 11: e0145092
- Bayer EM, Mongrand S, Tilsner J (2014) Specialized membrane domains of plasmodesmata, plant intercellular nanopores. **Front Plant Sci** 5: 507
- Ben-Tov D, Abraham Y, Stav S, Thompson K, Loraine A, Elbaum R, de Souza A, Pauly M, Kieber JJ, Harpaz-Saad S (2015) COBRA-LIKE2, a member of the glycosylphosphatidylinositol-anchored COBRA-LIKE family, plays a role in cellulose deposition in arabidopsis seed coat mucilage secretory cells. **Plant Physiol** 167: 711-724
- Benitez-Alfonso Y, Faulkner C, Pendle A, Miyashima S, Helariutta Y and Maule A (2013) Symplastic intercellular connectivity regulates lateral root patterning. **Dev Cell** 26: 136-147
- Bonnon C, Wendeler MW, Paccaud JP, Hauri HP (2010) Selective export of human GPI-anchored proteins from the endoplasmic reticulum. **J Cell Sci** 123: 1705-1715
- Bordier C, Etges RJ, Ward J, Turner MJ, Cardoso de Almeida ML (1986) Leishmania and Trypanosoma surface glycoproteins have a common glycopospholipid membrane anchor. **Proc Natl Acad Sci USA** 83: 5988-5991
- Borner GH, Lilley KS, Stevens TJ, Dupree P (2003) Identification of glycosylphosphatidylinositol-anchored proteins in *Arabidopsis*. A proteomic and genomic analysis. **Plant Physiol** 132: 568-577
- Borner GH, Sherrier DJ, Weimar T, Michaelson LV, Hawkins ND, Macaskill A, Napier JA, Beale MH, Lilley KS, Dupree P (2005) Analysis of detergent-resistant membranes in *Arabidopsis*. Evidence for plasma membrane lipid rafts. **Plant Physiol** 137: 104-116
- Bowman SM, Piwowar A, Al Dabbous M, Vierula J, Free SJ (2006) Mutational analysis of the glycosylphosphatidylinositol (GPI) anchor pathway demonstrates that GPI-anchored proteins are required for cell wall biogenesis and normal hyphal growth in *Neurospora crassa*. **Eukaryot Cell** 5: 587-600

Brown DM, Zeef LA, Ellis J, Goodacre R and Turner SR (2005) Identification of novel genes in *Arabidopsis* involved in secondary cell wall formation using expression profiling and reverse genetics. **Plant Cell** 17: 2281-2295

Bundy MG, Kosentka PZ, Willet AH, Zhang L, Miller E and Shpak ED (2016) A mutation in the catalytic subunit of the glycosylphosphatidylinositol transamidase disrupts growth, fertility, and stomata formation. **Plant Physiol** 171: 974-985

Cacas JL, Bure C, Grosjean K, Gerbeau-Pissot P, Lherminier J, Rombouts Y, Maes E, Bossard C, Gronnier J, Furt F, Fouillen L, Germain V, Bayer E, Cluzet S, Robert F, Schmitter JM, Deleu M, Lins L, Simon-Plas F, Mongrand S (2016) Revisiting plant plasma membrane lipids in tobacco: A focus on sphingolipids. **Plant Physiol** 170: 367-384

Cagnola JJ, Dumont de Chassart GJ, Ibarra SE, Chimenti C, Ricardi MM, Delzer B, Ghiglione H, Zhu T, Otegui ME, Estevez JM, Casal JJ (2018) Reduced expression of selected Fasciclin-Like arabinogalactan protein genes associates with the abortion of kernels in field crops of *Zea mays* (maize) and of *Arabidopsis* seeds. **Plant Cell Environ** 41: 661-674

Capron A, Gourgues M, Neiva LS, Faure JE, Berger F, Pagnussat G, Krishnan A, Alvarez-Mejia C, Vielle-Calzada JP, Lee YR, Liu B, Sundaresan V (2008) Maternal control of male-gamete delivery in *Arabidopsis* involves a putative GPI-anchored protein encoded by the *LORELEI* gene. **Plant Cell** 20: 3038-3049

Castillon GA, Aguilera-Romero A, Manzano-Lopez J, Epstein S, Kajiwarra K, Funato K, Watanabe R, Riezman H, Muniz M (2011) The yeast p24 complex regulates GPI-anchored protein transport and quality control by monitoring anchor remodeling. **Mol Biol Cell** 22: 2924-2936

Cermak T, Curtin SJ, Gil-Humanes J, Cegan R, Kono TJY, Konecna E, Belanto JJ, Starker CG, Mathre JW, Greenstein RL, Voytas DF (2017) A multipurpose toolkit to enable advanced genome engineering in plants. **Plant Cell** 29: 1196-1217

Ching A, Dhugga KS, Appenzeller L, Meeley R, Bourett TM, Howard RJ, Rafalski A (2006) Brittle stalk 2 encodes a putative glycosylphosphatidylinositol-anchored protein that affects mechanical strength of maize tissues by altering the composition and structure of secondary cell walls. **Planta** 224: 1174-1184

Coimbra S, Costa M, Jones B, Mendes MA, Pereira LG (2009) Pollen grain development is compromised in *Arabidopsis* agp6 agp11 null mutants. **J Exp Bot** 60: 3133-3142

Conzelmann A, Riezman H, Desponds C, Bron C (1988) A major 125-kd membrane glycoprotein of *Saccharomyces cerevisiae* is attached to the lipid bilayer through an inositol-containing phospholipid. **EMBO J** 7: 2233-2240

Coppinger P, Repetti PP, Day B, Dahlbeck D, Mehler A, Staskawicz BJ (2004) Overexpression of the plasma membrane-localized NDR1 protein results in enhanced bacterial disease resistance in *Arabidopsis thaliana*. **Plant J** 40: 225-237

Costa ML, Sobral R, Ribeiro Costa MM, Amorim MI, Coimbra S (2015) Evaluation of the presence of arabinogalactan proteins and pectins during *Quercus suber* male gametogenesis. **Ann Bot** 115: 81-92

Dai X, You C, Chen G, Li X, Zhang Q, Wu C (2011) OsBC1L4 encodes a COBRA-like protein that affects cellulose synthesis in rice. **Plant Mol Biol** 75: 333-345

Dai XR, Gao XQ, Chen GH, Tang LL, Wang H, Zhang XS (2014) Abnormal pollen tube guidance1, an Endoplasmic Reticulum-Localized Mannosyltransferase Homolog of glycosylphosphatidylinositol10 in Yeast and phosphatidylinositol glycan anchor biosynthesis B in Human, is required for *Arabidopsis* pollen tube micropylar guidance and embryo development. **Plant Physiol** 165: 1544-1556

De Caroli M, Lenucci MS, Di Sansebastiano GP, Dalessandro G, De Lorenzo G, Piro G (2011) Protein trafficking to the cell wall occurs through mechanisms distinguishable from default sorting in tobacco. **Plant J** 65: 295-308

Debono A, Yeats TH, Rose JK, Bird D, Jetter R, Kunst L, Samuels L (2009) Arabidopsis LTPG is a glycosylphosphatidylinositol-anchored lipid transfer protein required for export of lipids to the plant surface. **Plant Cell** 21: 1230-1238

Demesa-Arevalo E, Vielle-Calzada JP (2013) The classical arabinogalactan protein AGP18 mediates megaspore selection in *Arabidopsis*. **Plant Cell** 25: 1274-1287

Domozych DS, Popper ZA, Sorensen I (2016) Charophytes: Evolutionary giants and emerging model organisms. **Front Plant Sci** 7: 1470

Edstam MM, Edqvist J (2014) Involvement of GPI-anchored lipid transfer proteins in the development of seed coats and pollen in *Arabidopsis thaliana*. **Physiol Plant** 152: 32-42

Edstam MM, Laurila M, Hoglund A, Raman A, Dahlstrom KM, Salminen TA, Edqvist J, Blomqvist K (2014) Characterization of the GPI-anchored lipid transfer proteins in the moss *Physcomitrella patens*. **Plant Physiol Biochem** 75: 55-69

Eichler J, Adams MW (2005) Posttranslational protein modification in Archaea. **Microbiol Mol Biol Rev** 69: 393-425

Eisenhaber B, Bork P, Eisenhaber F (1999) Prediction of potential GPI-modification sites in proprotein sequences. **J Mol Biol** 292: 741-758

Ellis M, Egelund J, Schultz CJ, Basic A (2010) Arabinogalactan-proteins: key regulators at the cell surface? **Plant Physiol** 153: 403-419

Elortza F, Mohammed S, Bunkenborg J, Foster LJ, Nuhse TS, Brodbeck U, Peck SC, Jensen ON (2006) Modification-specific proteomics of plasma membrane proteins: Identification and characterization of glycosylphosphatidylinositol-anchored proteins released upon phospholipase D treatment. **J Proteome Res** 5: 935-943

Engelsdorf T, Will C, Hofmann J, Schmitt C, Merritt BB, Rieger L, Frenger MS, Marschall A, Franke RB, Pattathil S, Voll LM (2017) Cell wall composition and penetration resistance against the fungal pathogen *Colletotrichum higginsianum* are affected by impaired starch turnover in *Arabidopsis* mutants. **J Exp Bot** 68: 701-713

Fankhauser N, Maser P (2005) Identification of GPI anchor attachment signals by a Kohonen self-organizing map. **Bioinformatics** 21: 1846-1852

Faulkner C, Petutschnig E, Benitez-Alfonso Y, Beck M, Robatzek S, Lipka V, Maule AJ (2013) LYM2-dependent chitin perception limits molecular flux via plasmodesmata. **Proc Natl Acad Sci USA** 110: 9166-9170

Fernandez-Calvino L, Faulkner C, Walshaw J, Saalbach G, Bayer E, Benitez-Alfonso Y, Maule A (2011) *Arabidopsis* plasmodesmal proteome. **PLoS One** 6: e18880

Fleet GH, Manners DJ (1977) The enzymic degradation of an alkali-soluble glucan from the cell walls of *Saccharomyces cerevisiae*. **J Gen Microbiol** 98: 315-327

Frankova L, Fry SC (2013) Biochemistry and physiological roles of enzymes that 'cut and paste' plant cell-wall polysaccharides. **J Exp Bot** 64: 3519-3550

Free SJ (2013) Fungal cell wall organization and biosynthesis. **Adv Genet** 81: 33-82

Fujihara Y, Ikawa M (2016) GPI-AP release in cellular, developmental, and reproductive biology. **J Lipid Res** 57: 538-545

Fujii T, Shimoi H, Imura Y (1999) Structure of the glucan-binding sugar chain of Tip1p, a cell wall protein of *Saccharomyces cerevisiae*. **Biochim Biophys Acta** 1427: 133-144

Fujita M, Kinoshita T (2012) GPI-anchor remodeling: Potential functions of GPI-anchors in intracellular trafficking and membrane dynamics. **Biochim Biophys Acta** 1821: 1050-1058

Fujita M, Watanabe R, Jaensch N, Romanova-Michaelides M, Satoh T, Kato M, Riezman H, Yamaguchi Y, Maeda Y, Kinoshita T (2011) Sorting of GPI-anchored proteins into ER exit sites by p24 proteins is dependent on remodeled GPI. **J Cell Biol** 194: 61-75

Gao H, Li R, Guo Y (2017a) *Arabidopsis* aspartic proteases A36 and A39 play roles in plant reproduction. **Plant Signal Behav** 12: e1304343

Gao H, Zhang Y, Wang W, Zhao K, Liu C, Bai L, Li R, Guo Y (2017b) Two membrane-anchored aspartic proteases contribute to pollen and ovule development. **Plant Physiol** 173: 219-239

Gao Y, Bian L, Shi J, Xu J, Xi M, Wang G (2013) Expression of a conifer COBRA-like gene ClCOBL1 from Chinese fir (*Cunninghamia lanceolata*) alters the leaf architecture in tobacco. **Plant Physiol Biochem** 70: 483-491

Gaspar YM, Nam J, Schultz CJ, Lee LY, Gilson PR, Gelvin SB, Bacic A (2004) Characterization of the *Arabidopsis* lysine-rich arabinogalactan-protein AtAGP17 mutant (rat1) that results in a decreased efficiency of agrobacterium transformation. **Plant Physiol** 135: 2162-2171

Gillmor CS, Lukowitz W, Brininstool G, Sedbrook JC, Hamann T, Poindexter P, Somerville C (2005) Glycosylphosphatidylinositol-anchored proteins are required for cell wall synthesis and morphogenesis in *Arabidopsis*. **Plant Cell** 17: 1128-1140

Grisson MS, Brocard L, Fouillen L, Nicolas W, Wewer V, Dormann P, Nacir H, Benitez-Alfonso Y, Claverol S, Germain V, Boutte Y, Mongrand S, Bayer EM (2015) Specific membrane lipid composition is important for plasmodesmata function in *Arabidopsis*. **Plant Cell** 27: 1228-1250

Gritsch C, Wan Y, Mitchell RA, Shewry PR, Hanley SJ, Karp A (2015) G-fibre cell wall development in willow stems during tension wood induction. **J Exp Bot** 66: 6447-6459

Hayashi S, Ishii T, Matsunaga T, Tominaga R, Kuromori T, Wada T, Shinozaki K, Hirayama T (2008) The glycerophosphoryl diester phosphodiesterase-like proteins SHV3 and its homologs play important roles in cell wall organization. **Plant Cell Physiol** 49: 1522-1535

Hemsley PA (2015) The importance of lipid modified proteins in plants. **New Phytol** 205: 476-489

Hemsley PA, Weimar T, Lilley KS, Dupree P, Grierson CS (2013) A proteomic approach identifies many novel palmitoylated proteins in *Arabidopsis*. **New Phytol** 197: 805-814

Hepler PK, Rounds CM, Winship LJ (2013) Control of cell wall extensibility during pollen tube growth. **Mol Plant** 6: 998-1017

Hou Y, Guo X, Cyprys P, Zhang Y, Bleckmann A, Cai L, Huang Q, Luo Y, Gu H, Dresselhaus T, Dong J, Qu LJ (2016) Maternal ENODLs are required for pollen tube reception in *Arabidopsis*. **Curr Biol** 26: 2343-2350

Huck N, Moore JM, Federer M, Grossniklaus U (2003) The *Arabidopsis* mutant *feronia* disrupts the female gametophytic control of pollen tube reception. **Development** 130: 2149-2159

Ischebeck T (2016) Lipids in pollen - They are different. **Biochim Biophys Acta** 1861: 1315-1328

Iswanto AB, Kim JY (2017) Lipid raft, regulator of plasmodesmal callose homeostasis. **Plants (Basel)** 6:

Jarsch IK, Konrad SS, Stratil TF, Urbanus SL, Szymanski W, Braun P, Braun KH and Ott T (2014) Plasma membranes are subcompartmentalized into a plethora of coexisting and diverse microdomains in *Arabidopsis* and *Nicotiana benthamiana*. **Plant Cell** 26: 1698-1711

Jeon BW, Hwang JU, Hwang Y, Song WY, Fu Y, Gu Y, Bao F, Cho D, Kwak JM, Yang Z, Lee Y (2008) The *Arabidopsis* small G protein ROP2 is activated by light in guard cells and inhibits light-induced stomatal opening. **Plant Cell** 20: 75-87

Johnson KL, Cassin AM, Lonsdale A, Wong GK, Soltis DE, Miles NW, Melkonian M, Melkonian B, Deyholos MK, Leebens-Mack J, Rothfels CJ, Stevenson DW, Graham SW, Wang X, Wu S, Pires JC, Edger PP, Carpenter EJ, Bacic A, Doblin MS, Schultz CJ (2017) Insights into the evolution of hydroxyproline-rich glycoproteins from 1000 plant transcriptomes. **Plant Physiol** 174: 904-921

Johnson KL, Kibble NA, Bacic A, Schultz CJ (2011) A fasciclin-like arabinogalactan-protein (FLA) mutant of *Arabidopsis thaliana*, *fla1*, shows defects in shoot regeneration. **PLoS ONE** 6: e25154

Jones MA, Raymond MJ, Smirnov N (2006) Analysis of the root-hair morphogenesis transcriptome reveals the molecular identity of six genes with roles in root-hair development in *Arabidopsis*. **Plant J** 45: 83-100

Kapteyn JC, Ram AF, Groos EM, Kollar R, Montijn RC, Van Den Ende H, Llobell A, Cabib E and Klis FM (1997) Altered extent of cross-linking of beta1,6-glucosylated mannoproteins to chitin in *Saccharomyces cerevisiae* mutants with reduced cell wall beta1,3-glucan content. **J Bacteriol** 179: 6279-6284

Kapteyn JC, Van Den Ende H, Klis FM (1999) The contribution of cell wall proteins to the organization of the yeast cell wall. **Biochim Biophys Acta** 1426: 373-383

Kaur R, Ma B, Cormack BP (2007) A family of glycosylphosphatidylinositol-linked aspartyl proteases is required for virulence of *Candida glabrata*. **Proc Natl Acad Sci USA** 104: 7628-7633

Kieliszewski MJ, Shpak E (2001) Synthetic genes for the elucidation of glycosylation codes for arabinogalactan-proteins and other hydroxyproline-rich glycoproteins. **Cell Mol Life Sci** 58: 1386-1398

Kierszniowska S, Seiwert B, Schulze WX (2009) Definition of *Arabidopsis* sterol-rich membrane microdomains by differential treatment with methyl-beta-cyclodextrin and quantitative proteomics. **Mol Cell Proteomics** 8: 612-623

Kim H, Lee SB, Kim HJ, Min MK, Hwang I, Suh MC (2012) Characterization of glycosylphosphatidylinositol-anchored lipid transfer protein 2 (LTPG2) and overlapping function between LTPG/LTPG1 and LTPG2 in cuticular wax export or accumulation in *Arabidopsis thaliana*. **Plant Cell Physiol** 53: 1391-1403

Kinoshita T (2014) Enzymatic mechanism of GPI anchor attachment clarified. **Cell Cycle** 13: 1838-1839

Kinoshita T, Fujita M (2016) Biosynthesis of GPI-anchored proteins: Special emphasis on GPI lipid remodeling. **J Lipid Res** 57: 6-24

Kitagaki H, Wu H, Shimoi H, Ito K (2002) Two homologous genes, DCW1 (YKL046c) and DFG5, are essential for cell growth and encode glycosylphosphatidylinositol (GPI)-anchored membrane proteins required for cell wall biogenesis in *Saccharomyces cerevisiae*. **Mol Microbiol** 46: 1011-1022

Kjellbom P, Snogerup L, Stohr C, Reuzeau C, McCabe PF, Pennell RI (1997) Oxidative cross-linking of plasma membrane arabinogalactan proteins. **Plant J** 12: 1189-1196

Knox JP, Benitez-Alfonso Y (2014) Roles and regulation of plant cell walls surrounding plasmodesmata. **Curr Opin Plant Biol** 22: 93-100

Kollar R, Reinhold BB, Petrakova E, Yeh HJ, Ashwell G, Drgonova J, Kapteyn JC, Klis FM, Cabib E (1997) Architecture of the yeast cell wall. Beta(1-->6)-glucan interconnects mannoprotein, beta(1-->3)-glucan, and chitin. **J Biol Chem** 272: 17762-17775

Konrad SS, Ott T (2015) Molecular principles of membrane microdomain targeting in plants. **Trends Plant Sci** 20: 351-361

Kryan DJ, Ting EL, Abeijon C, Kroos L, Fuller RS (2005) Yapsins are a family of aspartyl proteases required for cell wall integrity in *Saccharomyces cerevisiae*. **Eukaryot Cell** 4: 1364-1374

Kumar M, Wightman R, Atanassov I, Gupta A, Hurst CH, Hemsley PA, Turner S (2016) S-Acylation of the cellulose synthase complex is essential for its plasma membrane localization. **Science** 353: 166-169

Kunze M, Riedel J, Lange U, Hurwitz R, Tischner R (1997) Evidence for the presence of GPI-anchored PM-NR in leaves of *Beta vulgaris* and for PM-NR in barley leaves. **Plant Physiol Biochem** 35: 507-512

Lalanne E, Honys D, Johnson A, Borner GH, Lilley KS, Dupree P, Grossniklaus U, Twell D (2004) SETH1 and SETH2, two components of the glycosylphosphatidylinositol anchor biosynthetic pathway, are required for pollen germination and tube growth in *Arabidopsis*. **Plant Cell** 16: 229-240

Lee GH, Fujita M, Takaoka K, Murakami Y, Fujihara Y, Kanzawa N, Murakami K, Kajikawa E, Takada Y, Saito K, Ikawa M, Hamada H, Maeda Y, Kinoshita T (2016a) A GPI processing phospholipase A2, PGAP6, modulates Nodal signaling in embryos by shedding CRIPTO. **J Cell Biol** 215: 705-718

Lee SC, Knowles TJ, Postis VLG, Jamshad M, Parslow RA, Lin YP, Goldman A, Sridhar P, Overduin M, Muench SP, Dafforn TR (2016b) A method for detergent-free isolation of membrane proteins in their local lipid environment. **Nat Protocols** 11: 1149-1162

Levy A, Erlanger M, Rosenthal M, Epel BL (2007) A plasmodesmata-associated beta-1,3-glucanase in *Arabidopsis*. **Plant J** 49: 669-682

Li C, Yeh FL, Cheung AY, Duan Q, Kita D, Liu MC, Maman J, Luu EJ, Wu BW, Gates L, Jalal M, Kwong A, Carpenter H, Wu HM (2015) Glycosylphosphatidylinositol-anchored proteins as chaperones and co-receptors for FERONIA receptor kinase signaling in *Arabidopsis*. **Elife** 4:

Li J, Yu M, Geng LL, Zhao J (2010) The fasciclin-like arabinogalactan protein gene, FLA3, is involved in microspore development of *Arabidopsis*. **Plant J** 64: 482-497

Li S, Ge FR, Xu M, Zhao XY, Huang GQ, Zhou LZ, Wang JG, Kombrink A, McCormick S, Zhang XS, Zhang Y (2013) *Arabidopsis* COBRA-LIKE 10, a GPI-anchored protein, mediates directional growth of pollen tubes. **Plant J** 74: 486-497

Li Y, Qian Q, Zhou Y, Yan M, Sun L, Zhang M, Fu Z, Wang Y, Han B, Pang X, Chen M, Li J (2003) BRITTLE CULM1, which encodes a COBRA-like protein, affects the mechanical properties of rice plants. **Plant Cell** 15: 2020-2031

Lisanti MP, Sargiacomo M, Graeve L, Saltiel AR, Rodriguez-Boulan E (1988) Polarized apical distribution of glycosyl-phosphatidylinositol-anchored proteins in a renal epithelial cell line. **Proc Natl Acad Sci USA** 85: 9557-9561

Liu L, Shang-Guan K, Zhang B, Liu X, Yan M, Zhang L, Shi Y, Zhang M, Qian Q, Li J, Zhou Y (2013) Brittle Culm1, a COBRA-like protein, functions in cellulose assembly through binding cellulose microfibrils. **PLoS Genet** 9: e1003704

Liu X, Castro C, Wang Y, Noble J, Ponvert N, Bundy M, Hoel C, Shpak E, Palanivelu R (2016) The role of LORELEI in pollen tube reception at the interface of the synergid cell and pollen tube requires the modified eight-cysteine motif and the receptor-like kinase FERONIA. **Plant Cell** 28: 1035-1052

Low MG (1989) The glycosyl-phosphatidylinositol anchor of membrane-proteins. **Biochim Biophys Acta** 988: 427-454

Luschnig C, Seifert GJ (2011) Posttranslational modifications of plasma membrane proteins and their implications for plant growth and development. In: Murphy AS, Peer WA and Schulz B, eds. *The Plant Plasma Membrane*. Springer-Verlag Berlin Heidelberg. pp. 109

Ma Y, Yan C, Li H, Wu W, Liu Y, Wang Y, Chen Q, Ma H (2017) Bioinformatics prediction and evolution analysis of arabinogalactan proteins in the plant kingdom. **Front Plant Sci** 8: 66

MacMillan CP, Mansfield SD, Stachurski ZH, Evans R, Southerton SG (2010) Fasciclin-like arabinogalactan proteins: Specialization for stem biomechanics and cell wall architecture in *Arabidopsis* and *Eucalyptus*. **Plant J** 62: 689-703

MacMillan CP, Taylor L, Bi Y, Southerton SG, Evans R, Spokevicius A (2015) The fasciclin-like arabinogalactan protein family of *Eucalyptus grandis* contains members that impact wood biology and biomechanics. **New Phytol** 206: 1314-1327

Markham JE, Lynch DV, Napier JA, Dunn TM, Cahoon EB (2013) Plant sphingolipids: Function follows form. **Curr Opin Plant Biol** 16: 350-357

Martinez-Nunez L, Riquelme M (2015) Role of BGT-1 and BGT-2, two predicted GPI-anchored glycoside hydrolases/glycosyltransferases, in cell wall remodeling in *Neurospora crassa*. **Fungal Genet Biol** 85: 58-70

Matas-Rico E, van Veen M, Leyton-Puig D, van den Berg J, Koster J, Kedziora KM, Molenaar B, Weerts MJA, de Rink I, Medema RH, Giepmans BNG, Perrakis A, Jalink K, Versteeg R, Moolenaar WH (2016) Glycerophosphodiesterase GDE2 promotes neuroblastoma differentiation through glypican release and is a marker of clinical outcome. **Cancer Cell** 30: 548-562

Mongrand S, Stanislas T, Bayer EM, Lherminier J, Simon-Plas F (2010) Membrane rafts in plant cells. **Trends Plant Sci** 15: 656-663

Montesinos JC, Pastor-Cantizano N, Robinson DG, Marcote MJ, Aniento F (2014) *Arabidopsis* p24delta5 and p24delta9 facilitate Coat Protein I-dependent transport of the K/HDEL receptor ERD2 from the Golgi to the endoplasmic reticulum. **Plant J** 80: 1014-1030

- Montesinos JC, Sturm S, Langhans M, Hillmer S, Marcote MJ, Robinson DG, Aniento F (2012) Coupled transport of *Arabidopsis* p24 proteins at the ER-Golgi interface. **J Exp Bot** 63: 4243-4261
- Morel J, Claverol S, Mongrand S, Furt F, Fromentin J, Bessoule JJ, Blein JP, Simon-Plas F (2006) Proteomics of plant detergent-resistant membranes. **Mol Cell Proteomics** 5: 1396-1411
- Morita N, Nakazato H, Okuyama H, Kim Y, Thompson GA (1996) Evidence for a glycosylinositolphospholipid-anchored alkaline phosphatase in the aquatic plant *Spirodela oligorrhiza*. **Biochim Biophys Acta-General Subjects** 1290: 53-62
- Motose H, Sugiyama M, Fukuda H (2004) A proteoglycan mediates inductive interaction during plant vascular development. **Nature** 429: 873-878
- Muniz M, Riezman H (2000) Intracellular transport of GPI-anchored proteins. **EMBO J** 19: 10-15
- Muniz M, Zurzolo C (2014) Sorting of GPI-anchored proteins from yeast to mammals--common pathways at different sites? **J Cell Sci** 127: 2793-2801
- Niu E, Shang X, Cheng C, Bao J, Zeng Y, Cai C, Du X, Guo W (2015) Comprehensive analysis of the COBRA-Like (COBL) gene family in *Gossypium* identifies two COBLs potentially associated with fiber quality. **PLoS ONE** 10: e0145725
- Oxley D, Bacic A (1999) Structure of the glycosylphosphatidylinositol anchor of an arabinogalactan protein from *Pyrus communis* suspension-cultured cells. **Proc Natl Acad Sci US A** 96: 14246-14251
- Paladino S, Pocard T, Catino MA, Zurzolo C (2006) GPI-anchored proteins are directly targeted to the apical surface in fully polarized MDCK cells. **J Cell Biol** 172: 1023-1034
- Park S, Lee C, Sabharwal P, Zhang M, Meyers CLF, Sockanathan S (2013) GDE2 Promotes neurogenesis by glycosylphosphatidylinositol-anchor cleavage of RECK. **Science** 339: 324-328
- Pereira AM, Lopes AL, Coimbra S (2016) JAGGER, an AGP essential for persistent synergid degeneration and polytubey block in *Arabidopsis*. **Plant Signal Behav** 11: e1209616
- Petersen TN, Brunak S, von Heijne G, Nielsen H (2011) SignalP 4.0: Discriminating signal peptides from transmembrane regions. **Nat Meth** 8: 785-786
- Pielsticker LK, Mann KJ, Lin WL, Seivler D (2005) Raft-like membrane domains contain enzymatic activities involved in the synthesis of mammalian glycosylphosphatidylinositol anchor intermediates. **Biochem Biophys Res Commun** 330: 163-171

- Pierleoni A, Martelli PL, Casadio R (2008) PredGPI: A GPI-anchor predictor. **BMC Bioinfo** 9: Pittet M, Conzelmann A (2007) Biosynthesis and function of GPI proteins in the yeast *Saccharomyces cerevisiae*. **Biochim Biophys Acta** 1771: 405-420
- Poisson G, Chauve C, Chen X, Bergeron A (2007) FragAnchor: A large-scale predictor of glycosylphosphatidylinositol anchors in eukaryote protein sequences by qualitative scoring. **Genomics Proteomics Bioinfo** 5: 121-130
- Poulsen CP, Dilokpimol A, Geshi N (2015) Arabinogalactan biosynthesis: Implication of AtGALT29A enzyme activity regulated by phosphorylation and co-localized enzymes for nucleotide sugar metabolism in the compartments outside of the Golgi apparatus. **Plant Signal Behav** 10: e984524
- Poulsen CP, Dilokpimol A, Mouille G, Burow M, Geshi N (2014) Arabinogalactan glycosyltransferases target to a unique subcellular compartment that may function in unconventional secretion in plants. **Traffic** 15: 1219-1234
- Raffaele S, Bayer E, Lafarge D, Cluzet S, German Retana S, Boubekur T, Leborgne-Castel N, Carde JP, Lherminier J, Noirod E, Satiat-Jeunemaitre B, Laroche-Traineau J, Moreau P, Ott T, Maule AJ, Reymond P, Simon-Plas F, Farmer EE, Bessoule JJ, Mongrand S (2009) Remorin, a solanaceae protein resident in membrane rafts and plasmodesmata, impairs potato virus X movement. **Plant Cell** 21: 1541-1555
- Schindelman G, Morikami A, Jung J, Baskin TI, Carpita NC, Derbyshire P, McCann MC, Benfey PN (2001) COBRA encodes a putative GPI-anchored protein, which is polarly localized and necessary for oriented cell expansion in *Arabidopsis*. **Genes Dev** 15: 1115-1127
- Sedbrook JC, Carroll KL, Hung KF, Masson PH, Somerville CR (2002) The *Arabidopsis* *SKU5* gene encodes an extracellular glycosyl phosphatidylinositol-anchored glycoprotein involved in directional root growth. **Plant Cell** 14: 1635-1648
- Shen Q, Bourdais G, Pan H, Robatzek S, Tang D (2017) *Arabidopsis* glycosylphosphatidylinositol-anchored protein LLG1 associates with and modulates FLS2 to regulate innate immunity. **Proc Natl Acad Sci USA** 114: 5749-5754
- Shi H, Kim Y, Guo Y, Stevenson B, Zhu JK (2003) The *Arabidopsis* *SOS5* locus encodes a putative cell surface adhesion protein and is required for normal cell expansion. **Plant Cell** 15: 19-32
- Shinya T, Motoyama N, Ikeda A, Wada M, Kamiya K, Hayafune M, Kaku H, Shibuya N (2012) Functional characterization of CEBiP and CERK1 homologs in arabidopsis and rice reveals the presence of different chitin receptor systems in plants. **Plant Cell Physiol** 53: 1696-1706

Showalter AM, Basu D (2016) Glycosylation of arabinogalactan-proteins essential for development in *Arabidopsis*. **Commun Integr Biol** 9: e1177687

Simpson C, Thomas C, Findlay K, Bayer E, Maule AJ (2009) An *Arabidopsis* GPI-anchor plasmodesmal neck protein with callose binding activity and potential to regulate cell-to-cell trafficking. **Plant Cell** 21: 581-594

Sindhu A, Langewisch T, Olek A, Multani DS, McCann MC, Vermerris W, Carpita NC, Johal G (2007) Maize Brittle stalk2 encodes a COBRA-like protein expressed in early organ development but required for tissue flexibility at maturity. **Plant Physiol** 145: 1444-1459

Song H, Kim J, Cosman D, Choi I (2006) Soluble ULBP suppresses natural killer cell activity via down-regulating NKG2D expression. **Cell Immunol** 239: 22-30

Staudt E, Ramasamy P, Plattner H, Simon M (2016) Differential subcellular distribution of four phospholipase C isoforms and secretion of GPI-PLC activity. **Biochim Biophys Acta** 1858: 3157-3168

Svetek J, Yadav MP, Nothnagel EA (1999) Presence of a glycosylphosphatidylinositol lipid anchor on rose arabinogalactan proteins. **J Biol Chem** 274: 14724-14733

Takahashi D, Kawamura Y, Uemura M (2013) Detergent-resistant plasma membrane proteome to elucidate microdomain functions in plant cells. **Front Plant Sci** 4: 27

Takahashi D, Kawamura Y, Yamashita T, Uemura M (2012) Detergent-resistant plasma membrane proteome in oat and rye: Similarities and dissimilarities between two monocotyledonous plants. **J Proteome Res** 11: 1654-1665

Takos AM, Dry IB, Soole KL (1997) Detection of glycosyl-phosphatidylinositol-anchored proteins on the surface of *Nicotiana tabacum* protoplasts. **FEBS Lett** 405: 1-4

Tan L, Eberhard S, Pattathil S, Warder C, Glushka J, Yuan C, Hao Z, Zhu X, Avci U, Miller JS, Baldwin D, Pham C, Orlando R, Darvill A, Hahn MG, Kieliszewski MJ, Mohnen D (2013) An *Arabidopsis* cell wall proteoglycan consists of pectin and arabinoxylan covalently linked to an arabinogalactan protein. **Plant Cell** 25: 270-287

Tan L, Leykam JF, Kieliszewski MJ (2003) Glycosylation motifs that direct arabinogalactan addition to arabinogalactan-proteins. **Plant Physiol** 132: 1362-1369

Tapken W, Murphy AS (2015) Membrane nanodomains in plants: capturing form, function, and movement. **J Exp Bot** 66: 1573-1586

Titapiwatanakun B, Blakeslee JJ, Bandyopadhyay A, Yang H, Mravec J, Sauer M, Cheng Y, Adamec J, Nagashima A, Geisler M, Sakai T, Friml J, Peer WA, Murphy AS (2009) ABCB19/PGP19 stabilises PIN1 in membrane microdomains in *Arabidopsis*. **Plant J** 57: 27-44

Tjellstrom H, Hellgren LI, Wieslander A, Sandelius AS (2010) Lipid asymmetry in plant plasma membranes: phosphate deficiency-induced phospholipid replacement is restricted to the cytosolic leaflet. **FASEB J** 24: 1128-1138

Tsai YH, Liu X, Seeberger PH (2012) Chemical biology of glycosylphosphatidylinositol anchors. **Angew Chem Int Ed Engl** 51: 11438-11456

Udenfriend S, Kodukula K (1995) How glycosyl-phosphatidylinositol-anchored membrane proteins are made. **Annu Rev Biochem** 64: 563-591

Vaddepalli P, Fulton L, Wieland J, Wassmer K, Schaeffer M, Ranf S, Schneitz K (2017) The cell wall-localized atypical beta-1,3 glucanase ZERZAUST controls tissue morphogenesis in *Arabidopsis thaliana*. **Development** 144: 2259-2269

van de Meene AM, Doblin MS, Bacic A (2017) The plant secretory pathway seen through the lens of the cell wall. **Protoplasma** 254: 75-94

Vogel JP, Raab TK, Schiff C, Somerville SC (2002) PMR6, a pectate lyase-like gene required for powdery mildew susceptibility in *Arabidopsis*. **Plant Cell** 14: 2095-2106

Willmann R, Lajunen HM, Erbs G, Newman MA, Kolb D, Tsuda K, Katagiri F, Fliegmann J, Bono JJ, Cullimore JV, Jehle AK, Gotz F, Kulik A, Molinaro A, Lipka V, Gust AA, Nurnberger T (2011) Arabidopsis lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. **Proc Natl Acad Sci USA** 108: 19824-19829

Wolf S, Hofte H (2014) Growth control: A saga of cell walls, ROS, and peptide receptors. **Plant Cell** 26: 1848-1856

Xiang JJ, Zhang GH, Qian Q, Xue HW (2012) Semi-rolled leaf1 encodes a putative glycosylphosphatidylinositol-anchored protein and modulates rice leaf rolling by regulating the formation of bulliform cells. **Plant Physiol** 159: 1488-1500

Xue H, Veit C, Abas L, Tryfona T, Maresch D, Ricardi MM, Estevez JM, Strasser R, Seifert GJ (2017) *Arabidopsis thaliana* FLA4 functions as a glycan-stabilized soluble factor via its carboxy-proximal Fasciclin 1 domain. **Plant J** 91: 613-630

Yeats TH, Sorek H, Wemmer DE, Somerville CR (2016) Cellulose deficiency is enhanced on hyper accumulation of sucrose by a H⁺-coupled sucrose symporter. **Plant Physiol** 171: 110-124

Youl JJ, Basic A, Oxley D (1998) Arabinogalactan-proteins from *Nicotiana glauca* and *Pyrus communis* contain glycosylphosphatidylinositol membrane anchors. **Proc Natl Acad Sci USA** 95: 7921-7926

Zavaliev R, Dong X, Epel BL (2016) Glycosylphosphatidylinositol (GPI) modification serves as a primary plasmodesmal sorting signal. **Plant Physiol** 172: 1061-1073

Zhao ZD, Tan L, Showalter AM, Lamport DT, Kieliszewski MJ (2002) Tomato LeAGP-1 arabinogalactan-protein purified from transgenic tobacco corroborates the Hyp contiguity hypothesis. **Plant J** 31: 431-444

Zhong R, Ye ZH (2015) Secondary cell walls: Biosynthesis, patterned deposition and transcriptional regulation. **Plant Cell Physiol** 56: 195-214

Zurzolo C, Simons K (2016) Glycosylphosphatidylinositol-anchored proteins: Membrane organization and transport. **Biochim Biophys Acta** 1858: 632-639

Author Manuscript

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Yeats, TH;Bacic, A;Johnson, KL

Title:

Plant glycosylphosphatidylinositol anchored proteins at the plasma membrane-cell wall nexus

Date:

2018-08-01

Citation:

Yeats, T. H., Bacic, A. & Johnson, K. L. (2018). Plant glycosylphosphatidylinositol anchored proteins at the plasma membrane-cell wall nexus. JOURNAL OF INTEGRATIVE PLANT BIOLOGY, 60 (8), pp.649-669. <https://doi.org/10.1111/jipb.12659>.

Persistent Link:

<http://hdl.handle.net/11343/283902>